JUDGE SAND SERVICE

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

07 CV 3893

STRUCTURE PROBE INC.,

*Plaintiff*,

v.

EMS ACQUISITION CORP. (d/b/a, ELECTRON MICROSCOPY SCIENCES),

*Defendant*.

**COMPLAINT**

Case No.



**JURY TRIAL DEMANDED**

ECF CASE

Plaintiff STRUCTURE PROBE INC. ("SPI" or "Plaintiff") alleges the following:

## NATURE OF ACTION

1. This is an action seeking damages and injunctive relief based upon copyright infringement and unfair competition by EMS ACQUISITION CORP., a/k/a Electron Miscropy Sciences ("EMS" or "Defendant") in infringing, copying, and misappropriating protectible elements of Plaintiff's website in violation of the laws of the United States and the State of New York.

## THE PARTIES

2. SPI is a corporation organized and existing under the laws of Delaware with its principal place of business located at 569 East Gay Street, West Chester, Pennsylvania.

3. Defendant is a corporation organized and existing under the laws of the Pennsylvania with its principal place of business located at 1560 Industry Road, Hatfield, Pennsylvania.

## JURISDICTION AND VENUE

4. This action arises under the Copyright Act, 17 U.S.C. § 101 *et seq.*, § 43(a) of the Lanham Act, 15 U.S.C. § 1125(a), and the laws of the State of New York.

5. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338(a), and 1367.

6. This Court has personal jurisdiction over the Defendant in that it conducts business in the State of New York and in this District, including engaging in the sale, offers of sale, and the advertisement of the products accused herein.

7. Venue is proper in this District pursuant to 28 U.S.C. §§ 1391 and 1400, in that, *inter alia*, Defendant's unlawful activities as alleged below were and continue to be committed and had and continue to have a substantial impact in the State of New York and this District.

## FACTS

8. Plaintiff is a manufacturer, retailer and distributor of analytical equipment and supplies. Plaintiff markets, promotes and advertises its products through, *inter alia*, its website at www.2spi.com ("SPI Website"). Plaintiff is the creator of certain webpages, images, text, and artwork on the SPI Website, and is the owner of certain copyrights covering the webpages, images, text, and artwork of the SPI Website.

9. Plaintiff and Defendant are competitors. Upon information and belief, without Plaintiff's permission, Defendant has intentionally linked to the SPI Website from Defendant's website and thereby created confusion as to the source, origin, and sponsorship of products sold

through its website, www.emsdiasum.com ("EMS Website"), and created confusion as to a possible affiliation with Plaintiff.

10. Upon information and belief, without Plaintiff's permission, Defendant has willfully and systematically copied and continues to copy webpages and text from the SPI Website, including Plaintiff's copyrighted works.

11. For one (non-limiting example), upon information and belief, Defendant sells a product called EMS #12667 and markets it on the EMS Website through EMS's webpage entitled "Leit-C Plast" ("Leit-C Plast webpage"). In the Leit-C Plast webpage, Defendant copied portions of an SPI webpage entitled "LEIT-C-Plast™ Carbon Cement." Compare Exhibit A (SPI) with Exhibit B (EMS). Defendant copied Plaintiff's HTML source code, which is the computer code allowing browsers to display the SPI Website. Defendant's source code for the Leit-C Plast webpage contains an express link to SPI's Website as shown by references to a URL for the SPI Website. Exhibit C. The Leit-C Plast webpage contains embedded links to webpages of the SPI Website.

12. For another (non-limiting example), upon information and belief, Defendant sells a product called an EMS 1050 Plasma Asher and markets it on the EMS Website, through Defendant's webpage entitled "Plasma Asher: For Ashing, Etching & Cleaning" ("Plasma Asher webpage"). In the Plasma Asher webpage, Defendant copied portions of an SPI webpage entitled "SPI Plasma Prep™ II User's Manual." Compare Exhibit D (SPI) with Exhibit E (EMS). SPI is the owner of a United States copyright application covering SPI's Plasma Pre II User's Manual. Exhibit D.

13. Upon information and belief, Defendant has copied numerous additional webpages and text from the SPI Website. See, for example, Exhibits F, G, H, I, J, K, and L.

14. Upon information and belief, without Plaintiff's permission, Defendant has advertised and continues to advertise through its website various products using webpages which infringe upon Plaintiff's copyright.

**COUNT I**
**COPYRIGHT INFRINGEMENT**

15. Plaintiff incorporates herein by reference each and every allegation contained in each paragraph above.

16. Plaintiff is, and at all relevant times has been, the copyright owner under United States copyright with respect to "SPI Plasma Prep™ II User's Manual." A true and correct copy of the copyright application for this work is attached as Exhibit A hereto.

17. Plaintiff believes that Defendant, without permission or consent, has copied, distributed, advertised, and posted (and continues to copy, distribute, advertise, and post) works authored and owned by Plaintiff, including the Plasma Asher webpage, on the EMS Website. In doing so, Defendant violated and continues to violate Plaintiff's exclusive rights of reproduction and distribution under the Copyright Act.

18. Defendant's foregoing acts of infringement have been willful.

19. As a result of Defendant's infringement of Plaintiff's copyrights, Plaintiff is entitled to statutory damages pursuant to 17 U.S.C. § 504(c) for Defendant's infringement of each copyrighted work.

20. Plaintiff is entitled to disgorgement of all profits made by Defendant for each illegal sale of products advertised using works which infringement Plaintiff's copyrights, including the EMS 1050 Plasma Asher.

21. Plaintiff is entitled to its attorneys' fees and costs pursuant to 17 U.S.C. § 505.

22. The conduct of Defendant causes, and unless enjoined and restrained by this Court, will continue to cause Plaintiff irreparable injury that cannot fully be compensated or measured in money, and Plaintiff has no adequate remedy at law. Pursuant to 17 U.S.C. §§ 502 and 503, Plaintiff is entitled to injunctive relief prohibiting Defendant from further infringing Plaintiff's copyrights, and ordering Defendant to destroy all copies of the Plasma Asher webpage, marketing material, and any and all additional webpages or marketing materials (including catalogues) made in violation of Plaintiff's exclusive rights.

## COUNT II
## FEDERAL UNFAIR COMPETITION

23. Plaintiff incorporates herein by reference each and every allegation contained in each paragraph above.

24. Defendant has engaged in a series of deceptive commercial practices in an effort to obtain unfair gains and competitive advantages.

25. Defendant has, in connection with the commercial advertising and promotion of its products (*e.g.*, EMS #12667), falsely, misleadingly, and confusingly indicated Plaintiff's sponsorship, origin, and/or endorsement of its products by linking to the SPI Website. Additionally, by embedding links to the SPI Website, Defendant creates a likelihood of confusion by consumers, who may wrongly assume that Plaintiff sponsors or otherwise approves

Defendant's use of Plaintiff's trade dress, trademarks, and website and/or that Plaintiff is in some way affiliated with Defendant. Defendant has, in connection with the commercial advertising and promotion of its products (*e.g.*, EMS 1050 Plasma Asher), misrepresented the nature, characteristics and qualities of its products and failed to disclose that it has copied substantial portions of the SPI Website in the advertising of its products. In doing so, Defendant has violated § 43(a) of the Lanham Act, 15 U.S.C. § 1125.

26. Defendant's acts of unfair competition have caused and continue to cause Plaintiff to suffer injury.

27. The conduct of Defendant causes, and unless enjoined and restrained by this Court, will continue to cause Plaintiff irreparable injury that cannot fully be compensated or measured in money, and Plaintiff has no adequate remedy at law.

28. Defendant's conduct was willful, rendering this an exceptional case warranting the imposition of treble damages and award of Plaintiff's reasonable attorney's fees.

**COUNT III**
**NEW YORK UNFAIR COMPETITION**

29. Plaintiff incorporates herein by reference each and every allegation contained in each paragraph above.

30. Defendant has engaged in the misappropriation of Plaintiff's skill, expenditures, and labor.

31. Defendant has in bad faith, and in connection with the commercial advertising and promotion of its products, misappropriated the labors and expenditures of Plaintiff by

copying Plaintiff's works, including webpages, and text from the SPI Website. Defendant's conduct is likely to cause confusion or to deceive purchasers as to the origins of the products for which Defendant has copied Plaintiff's works. In doing so, Defendant violated New York laws of unfair competition and false advertising, including New York common law and New York Gen. Bus. Law §§ 349 and 350.

32. Defendant's acts of misappropriation of Plaintiff's skill, expenditures, and labor has caused and continue to cause Plaintiff to suffer injury. The conduct of Defendant is causing, and unless enjoined and restrained by this Court, will continue to cause Plaintiff irreparable injury that cannot fully be compensated or measured in money, and Plaintiff has no adequate remedy at law.

33. Defendant's misappropriation was willful.

**WHEREFORE**, Plaintiff respectfully requests the following relief:

A. Judgment for Plaintiff that Defendant has infringed Plaintiff's copyright and Defendant violated the Lanham Act and the unfair competition law of the State of New York;

B. An order preliminarily and permanently enjoining and restraining Defendant and its officers, agents, servants, employees, attorneys, and all persons in active concert or participation with any of them, from:

(i) copying Plaintiff's copyrighted work(s);

(ii) displaying, selling, and distributing any products that infringe Plaintiff's copyrighted work;

(iii) otherwise infringing or causing others to infringe Plaintiff's copyrighted work;

(iv) linking to Plaintiff's website without Plaintiff's permission;

(v) passing itself off as affiliated with Plaintiff; and

(vi) misappropriating the labor of Plaintiff by copying portions of its webpages.

C. An order awarding all damages that Plaintiffs have sustained and will sustain as a result of Defendant's infringing acts, and all gains, profits and advantages obtained by Defendant's infringing acts, and all gain, profits and advantages obtained by Defendant as a result, in an amount to be determined at trial;

D. An order awarding Plaintiff all other damages suffered by reason of Defendant's wrongful acts, including reasonable attorneys' fees, pursuant to 17 U.S.C. § 505 and/or 15 U.S.C. § 1117(a), and the cost of this action;

E. An order awarding Plaintiff statutory damages for Defendant's willful infringement;

F. An order awarding Plaintiff treble damages pursuant to 15 U.S.C. § 1117(b); and

G. An order awarding Plaintiff such other and further relief as the Court deems just and proper.

**JURY DEMAND**

Plaintiff demands trial by jury.

Dated: New York, New York
May 17, 2007

KENYON & KENYON LLP

Joseph F. Nicholson (7307)
Michael J. Freno (6969)
One Broadway
New York, New York 10004
212-425-7200

*Counsel for Plaintiff Structure Probe, Inc.*

# EXHIBIT A

# LEIT-C-Plast™ Carbon Cement

**Instructions for Use**

Leit-C-Plast™ is a special adhesive material for the preparation or large specimens for scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS).

**Characteristics:**

- High electrical conductivity
- Permanent plasticity
- Vacuum compatible/stable
- High adhesive strength
- Absent are any peaks that would show up in an EDS spectrum

**Instructions for Use:**

Leit-C-Plast is rolled and flattened between two plastic plates that are supplied with the kit. A small amount of Leit-C-Plast is separated with a spatula, applied to a sample mount (sometimes called the stub) and if necessary, further distributed with a glass rod.

The specimen to be examined is then pressed in to the adhesive material already applied to the sample mount. Specimens that are not conductive may immediately be either sputter or carbon coated, or if examination will be by FESEM, either chromium or osmium coated.

For reorientation on the specimen mount, or preservation of the specimen separated from the mount, the sample maybe removed by literally lifting it off of the adhesive. Small traces of Leit-C-Plast are easily removed by the use of alcohol.

Because of the permanent viscoelasticity of Leit-C-Plast, very large specimens can be mounted surrounded by a ring of Leit-C-Plast and then when appropriate, the large specimen can be reoriented on the mount without difficulty.

For the most precise work, sometimes a combination of Tempfix™ and Leit-C-Plast is recommended. With this protocol, the specimen if fixed to the surface of the sample mount with solvent-free low melting point Tempfix. The next step is to apply the electrically conductive Leit-C-Plast as a conductive bridge between the specimen and the Tempfix. The combination system can be inserted directly into the vacuum at this point because of their vacuum compatibility.

Thin strands of ether Leit-C-Plast or Tempfix can be drawn down to a fiber point, which can be used as a pointer for special features that will later be examined by SEM.

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ON-LINE To Ask a Question or Make a Comment

ON-LINE To Place an Order or Request a Quote

Search SPI Navigator Index

Return to:

- Other Adhesives Table of Contents
- Specimen Preparation Table of Contents
- SPI Supplies Catalog Table of Contents

- SPI Supplies Home Page

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Wednesday November 01, 2006



Worldwide Distributors, Representatives, and Agents 

# EXHIBIT B

## Technical Data Sheets



Electron Microscopy Sciences
P.O. Box 550
1560 Industry Road
Hatfield, PA 19440
Tel #: 215-412-8400
Fax #: 215-412-8450
Fax #: 215-412-8451
E-Mail: sgkcck@aol.com

# ➤ Leit-C Plast

### EMS #12667

Leit-C-Plast™ is a special adhesive material for the preparation or large specimens for scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS).

### Characteristics

- High electrical conductivity
- Permanent plasticity
- Vacuum compatible/stable
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For the most precise work, sometimes a combination of Tempfix™ and Leit-C-Plast is recommended. With this protocol, the specimen if fixed to the surface of the sample mount with solvent-free low melting point Tempfix. The next step is to apply the electrically conductive Leit-C-Plast as a conductive bridge between the specimen and the Tempfix. The combination system can be inserted directly into the vacuum at this point because of their vacuum compatibility.

Thin strands of ether Leit-C-Plast or Tempfix can be drawn down to a fiber point, which can be used as a pointer for special features that will later be examined by SEM.

### Online Ordering

Leit-C Plast is available online from the EMS Catalog. For ordering or product information, click here.

# EXHIBIT C

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is a special adhesive material for the preparation or large specimens for scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS).</P>
<H2>Characteristics</H2>
<UL>
<LI>High electrical conductivity
<LI>Permanent plasticity
<LI>Vacuum compatible/stable
<LI>High adhesive strength
<LI>Absent are any peaks that would show up in an EDS spectrum </LI></UL>
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is rolled and flattened between two plastic plates that are supplied with the kit. A small amount of Leit-C-Plast is separated with a spatula, applied to a sample mount (sometimes called the stub) and if necessary, further distributed with a glass rod.</P>
<P>The specimen to be examined is then pressed in to the adhesive material already applied to the sample mount. Specimens that are not conductive may immediately be either <A href="1http://www.2spi.com/catalog/instruments/sputter.html">sputter or carbon coated</A>, or if examination will be by FESEM, either <A href=2http://www.2spi.com/catalog/instruments/ch-coaters.shtml">chromium</A> or <A href="3http://www.2spi.com/catalog/osmi-coat.html">osmium coated</A>.</P>
<P>For reorientation on the specimen mount, or preservation of the specimen separated from the mount, the sample maybe removed by literally lifting it off of the adhesive. Small traces of Leit-C-Plast are easily removed by the use of alcohol.</P>
<P>Because of the permanent viscoelasticity of Leit-C-Plast, very large specimens can be mounted surrounded by a ring of Leit-C-Plast and then when appropriate, the large specimen can be reoriented on the mount without difficulty.</P>
<P>For the most precise work, sometimes a combination of <A href=4http://www.2spi.com/catalog/spec_prep/cond_adhes3.shtml#from-t">Tempfix™</A> and Leit-C-Plast is recommended. With this protocol, the specimen if fixed to the surface of the sample mount with solvent-free low melting point Tempfix. The next step is to apply the electrically conductive Leit-C-Plast as a conductive bridge between the specimen and the Tempfix. The combination system can be inserted directly into the vacuum at this point because of their vacuum compatibility.</P>
<P>Thin strands of ether Leit-C-Plast or Tempfix can be drawn down to a fiber point, which can be used as a pointer for special features that will later be examined by SEM.</P>

```
<H2>Online Ordering</H2>
<P><A
href="http://www.emsdiasum.com/microscopy/products/sem/colloidal.aspx#12667">Leit-C
Plast</A> is available online from the <SPAN class=chemicals>EMS</SPAN> <A
href="http://www.emsdiasum.com/microscopy/products/catalog.aspx">Catalog</A>.
For ordering or product information, <A
href="http://www.emsdiasum.com/microscopy/products/sem/colloidal.aspx#12667">click
here</A>. </P>
<P> </P></DIV>
<DIV id=siteInfo><IMG height=34 alt=logo22
src="Leit-C Plast Technical Data Sheet_files/logo22w.gif" width=22> <A
href="http://www.emsdiasum.com/microscopy/company/about.aspx">About Us</A> | <A
href="http://www.emsdiasum.com/microscopy/products/index.aspx">Product Index
</A>| <A href="http://www.emsdiasum.com/microscopy/company/privacy.aspx">Privacy
Policy</A> | <A
href="http://www.emsdiasum.com/microscopy/company/contact.aspx">Contact Us</A> |
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class=chemicals>M</SPAN>icroscopy <SPAN class=chemicals>S</SPAN>ciences
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Plasma Prep II User's Manual

PREVIOUS OR ALTERNATIVE TITLES ▼

PUBLICATION AS A CONTRIBUTION If this work was published as a contribution to a periodical, serial, or collection, give information about the collective work in which the contribution appeared. Title of Collective Work ▼

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Pseudonymous? ☐ Yes ☐ No
If the answer to either of these questions is "Yes," see detailed instructions.

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AUTHOR'S NATIONALITY OR DOMICILE Name of Country
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**3** a YEAR IN WHICH CREATION OF THIS WORK WAS COMPLETED This information must be given in all cases. 2006 ◀ Year

b DATE AND NATION OF FIRST PUBLICATION OF THIS PARTICULAR WORK Complete this information ONLY if this work has been published. Month ▶ December Day ▶ 14 Year ▶ 2006
United States ◀ Nation

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569 East Gay Street
West Chester, PA 19380

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Michael J. Freno
One Broadway
New York, NY 10004

Area code and daytime telephone number ▶ 212.425.7200 Fax number ▶ 212.425.5288

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of the work identified in this application and that the statements made by me in this application are correct to the best of my knowledge.

Typed or printed name and date ▼ If this application gives a date of publication in space 3, do not sign and submit it before that date.

Michael J. Freno, Esq. Date ▶ 4/18/07

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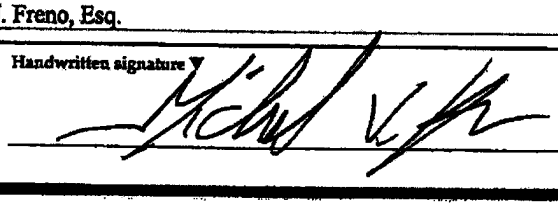

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Michael J. Freno

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# SPI Plasma Prep™ II User's Manual

**Section 1**

PLEASE - TAKE A MOMENT TO READ SECTION ONE [1] BEFORE PROCEEDING TO INSTALLATION
GENERAL DESCRIPTION - SPI PLASMA-PREP II?

## 1-1 GENERAL COMMENTS

The SPI Supplies Plasma-Prep II is a table top plasma chemistry reactor designed to provide plasma technology at a moderate cost. This simple-to-operate instrument can perform repeatable plasma chemical reactions with a minimum of automation. All controls are manual; however, where necessary, automatic monitors and controls take over to protect the equipment and the samples in the reactor. The Plasma-Prep II comes equipped with an internally housed RF generator. RF power is transferred from a power amplifier directly coupled to the reaction chamber. A variable capacitor provides a tuning control for resonating the power amplifier. An audible alarm sounds whenever the power amplifier is out of resonance. This also aids in tuning since the alarm-off "window" corresponds to the "in tune" state of the machine. Instrumentation for the Plasma-Prep II consists of a power tuning indicator and the audible tuning alarm.

## 1-2 FUNCTIONAL DESCRIPTION

The Plasma-Prep II is a small reactor which weighs under 31 pounds fully assembled, less vacuum pump. With the exception of the external vacuum pump, it is a fully self-contained machine. It consists of an RF generator and associated tuning circuits, a vacuum system with solenoid control valves, a constant feed gas supply system and a reaction chamber system which includes two semitubular electrodes and two Pyrex glass chamber elements.

### 1-2.1

RF Generator - The RF power source includes a solid state oscillator operating at 13.56 MHz, the FCC-authorized industrial frequency. A solid state driver and two 4-65A vacuum tube power amplifiers operated in parallel to conservatively provide a continuous wave power of 100 watts. Power transfer to the reaction chamber is accomplished via the electric field between the semitubular electrodes. Maximum power transfer is realized by a suitable tap on the final amplifier tuned circuit. The tuning control allows the tuning of the final amplifier tuned circuit to resonance providing the maximum output power.

### 1-2.2

Vacuum System - The vacuum system includes the vacuum pump (not supplied as part of the Plasma-Prep II), the vacuum hose, the vacuum valves and the control circuitry. The vacuum valve switch is interlocked with the RF generator switch to prevent the RF power from coming on unless the vacuum valve is energized. (CAUTION: There is no vacuum sensing, so the vacuum pump must be attached and operating).

### 1-2.3

Gas Supply System - The gas supply system for the Plasma-Prep II consists of the gas delivery system inside the reaction chamber. This delivery system is a glass tube sealed on the inner end and perforated along its bottom

surface. Connections to the delivery tube are made by sliding the silicone tubing over the barbed fittings on the glass chamber.

**1-2.4**

Reaction Chamber - The reaction chamber sub-system consists of an upper and lower electrode and a two element Pyrex glass reaction chamber (See Fig. 2-1). (For CF4 operation, a quartz chamber should be used). These are open ended cylinders designed to fit into each other to form a closed chamber. The chamber is sealed with a flat silicone gasket which seats against a raised lip on the inner chamber portion. The gas delivery tube feeds through the back of the outer chamber section. This chamber section also provides connection to the vacuum hose by a glass tube joined at the front of the chamber. The inner chamber is perforated by a series of slots located on the bottom surface of the chamber. Four little glass "feet" on the bottom of the inside chamber raise it off the inside surface of the outer chamber to provide a space between the two chamber sections. This arrangement provides for the best gas conduction flow and results in repeatable, dependable processing.

**1-2.5**

The AAS (Audio Alarm System) is provided to give an alarm when the plate dissipation of the final amplifier is excessive. Simultaneously, the AAS will disable the RF drive to the final amplifier and reduce this plate dissipation. This sets up a cyclical or pulsed mode. In this mode the RF power is turned on and off on a duty cycle that is inversely proportional to the plate dissipation. That is, the greater the dissipation, the shorter is the time the power is on. This will effectively protect the final amplifier tube's life, as excessive plate dissipation shortens tube life.

## 1-3 SAFETY INFORMATION

Interlocks - There are two microswitch interlocks engineered into the machine to prevent injury to operating personnel. These are:

a. Front door interlock-cuts off AC to the RF power supply

b. Right side, front and rear-shuts off all primary power

*****WARNING*****

**SINCE THE GAS MOST USUALLY USED IN THE PLASMA-PREP II IS OXYGEN, NO SMOKING SIGNS SHOULD BE POSTED NEAR THE MACHINE AND THE NO SMOKING BAN OBSERVED**

Even if a different gas is going to be used, we still recommend the enforcement of the NO SMOKING ban because, quite frequently, the user has an unanticipated need for oxygen and the NO SMOKING ban would be appropriate.

## 1-4 TYPICAL PLASMA PROCESS

The "Plasma Process" is accomplished through the use of a low pressure, RF induced gaseous discharge. The material or specimen is loaded into the reaction chamber. The chamber is evacuated to a mild vacuum (approximately 200 microns) by a mechanical vacuum pump. A carrier gas is drawn through the chamber over the specimen. Radio frequency power is applied around the chamber (at 13.56MHz). This excites the carrier gas molecules and changes some of them into chemically active atoms and molecules. The mechanism employed in this process is one of oxidation. Electrons produced by ionization of gas, gain energy in the electric field. Subsequent collisions between these energetic electrons and neutral gas molecules result in an

energy transfer to the molecules producing chemically active atoms, free radicals, ions and free electrons. The combustion products, which are completely dissociated and harmless, are carried away in the gas stream. The unique property of this process is that it occurs near ambient temperatures without employing toxic chemicals.

## 1-5 ACCESS TO PROCESS MONITORING

Another connection is provided for the addition of a pressure transducer to monitor chamber pressure. Recommended Model #11019 (see page 10). This connection is marked PRESS (Pressure) PORT. Spare parts are available (see page 25). TABLE OF SPECIFICATIONS

### PHYSICAL DIMENSIONS

| | |
|---|---|
| Height | 10.5 inches (26.7cm) |
| Width | 11.8 inches (30.0cm) |
| Length | 14.8 inches (37.6cm) |

### WEIGHT

| | |
|---|---|
| Assembled | 31 pounds |
| Ship w/container | 44 pounds |

### EFFECTIVE CHAMBER SIZE

Inside length 5.9 inches (15.0cm)

Inside diameter 4.15 inches (10.54cm)

| | |
|---|---|
| RF POWER | 0 to 100 Watts |
| RF FREQUENCY | 13.56MHz crystal controlled |
| RF TUNING | Variable capacitor to maintain resonance |
| AC POWER | 120 VAC, 50/60Hz 15 amp line service (not including vacuum pump) |

**NOTE: The Plasma-Prep II meets Article 18 of the FCC and NIOSH radiation standards.**

Return to:

- SPI Plasma Prep II Manual Table of Contents
- SPI Plasma-Prep II Plasma Etcher
- SPI Plasma Etcher Table of Contents
- SPI Supplies Catalog Table of Contents
- SPI Supplies Home Page

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Thursday December 14, 2006



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# EXHIBIT E

## Equipment

Online Catalog
- Chemicals
- ImmunoGold Reagents
- Embedding Media Kits
- TEM Grids and Supplies
- Silicone Nitride Films Mesh
- Microscope Calibration
- EM Accessories
- Microanalysis Standards
- Preparation & Embedding
- Materials & Metrology
- Histology Light Microscopy
- Scanning Electron, SEM
- Cryo Preparation
- Diatome Diamond Knives
- Photography
- Tweezers, Tools, Gadjets
- Microscopes, Graticles, Magnifiers, Illuminators
- Cleaning Supplies
- Pumps & Evaporation
- Equipment
- Safety Supplies
- Labels and Tape
- Lab Starter Kits
- Equipment Repair

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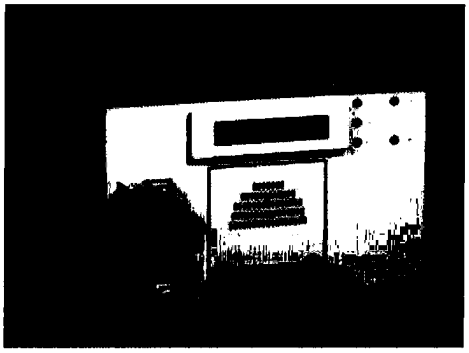
E-Mail: sgkcck@aol.com

Electron Microscopy Sciences

# Plasma Asher: For Ashing, Etching & Cleaning

## EMS 1050 Plasma Asher



### PLASMA ASHER: For Ashing, Etching & Cleaning

The Plasma process is accomplished through the use of a low pressure, RF induced gaseous discharge. The material or specimen is loaded into the reaction chamber. The chamber is evacuated to a vacuum pressure of 0.1-0.2 torr by a mechanical vacuum pump. A carrier gas is introduced into the chamber, raising the chamber pressure to 0.3-1.2 torr, depending on the application.

RF Power is applied around the chamber (13.56 MHz). This excites the carrier gas molecules and dissociates it into chemically active atoms and molecules. The mechanism employed in this process is one of ionization. The combustion products, which are completely dissociated and harmless are carried away in the gas stream. The unique property of this process is that it occurs near low temperatures without employing toxic chemicals.

The EMS 1050 consists of a solid state RF Generator and associated tuning circuits, a vacuum system with a solenoid controlled valve, a constant feed gas supply system, and a reaction chamber system which includes two semicircular electrodes and two piece pyrex chamber. The unit has one gas control as standard.

The solid state RF Generator is a solid state crystal controlled oscillator designed to provide up to 150 watts of continuous wave 13.56 MHz power to the reaction chamber. Maximum power transfer from the power supply to the reaction chamber is accomplished by matching the output impedance of the amplifier to the input impedance of the reaction chamber.

The gas supply system consists of the gas delivery system inside the reaction chamber. This delivery system is a glass tube sealed on the inner end and perforated along its bottom surface. Connections to the delivery tube are fastened with special clips to prevent the possible leakage of contaminants into the chamber.

The EMS 1050 is often used in Asbestos Specimen Preparation as a Low Temperature Ashing Technique.

### Features

Automatic tuning of RF power.
Built-in rotary vacuum pump.
Barrel chamber with isotropic etching.
Low temperature plasma ashing, etching, and cleaning (0-150 watts RF) .
Vacuum monitoring.
Dual flow gauge gas control.
Accurate process timer.
Needle value vent control.
Micro controller with default settings programmable by the operator.
Indication of settings by LCD display of status/entryIndication of conditions during cycle, vacuum, power, time.
Location bay for backing pump filled with special "oil"
Sample carrier for convenient loading.
Rack-out drawer loading door for ease of sample access.
Polycarbonate safety sheild

### Specifications

| | |
|---|---|
| Instrument Case | 450mm(W)x350mm(D)x300mm(H) |
| Barrel Work Chamber | [Pyrex] 150mm(L)x100mm(Dia.) |

| | |
|---|---|
| Barrel Work Chamber | Pyrex 150mm(L)x100mm(Dia.) (Borosilicate Glass as standard) |
| Weight | 25Kg |
| Plasma Output | Solid State Power Supply: 0-150 watts continuously variable at 13.56MHz with Tuning Control of forward and reflected power to optimize RF power transfer |
| Vacuum Gauge | >ATM to 1 x $10^{-5}$mbar Full scale normal 0.5 mbar to 1.0 mbar. |
| Digital Timer Unit | Displays elapsed time with range select: 0-99 min. 99 sec. 0-99 hours. Automatic termination of Ashing Process |
| Dual Gas Flow Gauge | Dual gas needle valve flow control selectable for 1, 2, or both gases (calibrated 5-100cm3/min air at A.T.P) |
| Supply | 115 Volts 60Hz (6 amp Max) 230 Volts 50Hz (3 amp Max) |
| Services | Process Gas at normal 5 psi (0.33 bar) |

CAUTION: For Oxygen or Corrosive Process Gases Vacuum Pump should use a Synthetic Oil 'Fomblin Oil', or similar

**Ordering Information**

| | | | |
|---|---|---|---|
| **93000** | EMS 1050 Plasma Asher | POR | Request Quote |
| **91005-F** | Rotary Vacuum Pump (Fomblin) | POR | Request Quote |

**SEM Cryogenic Preparation System**



Online Catalog
- Chemicals
- ImmunoGold Reagents
- Embedding Media Kits
- TEM Grids and Supplies
- Silicone Nitride Films Mesh
- Microscope Calibration
- EM Accessories
- Microanalysis Standards
- Preparation & Embedding
- Materials & Metrology
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Fax #: 215-412-8451
E-Mail: sgkcck@aol.com

# EXHIBIT F

# BioBond™ Tissue Adhesive

**Technical Data Sheet**

**Tissue Section Adhesive**
For immunohistochemistry the choice of specimen preparation is critical for the preservation of antigens in the sample. Of greatest importance in the preparation schedules are the specimen fixation and embedding. The protocol must satisfy the requirements for preservation of structural integrity and antigenicity.

**Why Coat Slides?**
Having prepared the tissue specimen for immunolabelling it is then imperative to perform the incubations with a protocol designed to maximise the specific signal and minimise the background. Some incubation conditions may cause tissue sections to be removed from the glass slide. Typical tissue section adhesives such as poly-L-Lysine, Elmer's glue, chrome alum, etc are not suitable for use with immunogold labelling because of the increased background caused by attraction of gold particles to the adhesive on the slide.

In addition the surface of glass slides is uneven and is activated by the silicon tetrahedral structure. It therefore provides active sites for absorption of proteins or reactions with chemicals and reagents. It is therefore important to minimise this possibility by coating the surface with a material that is of low reactivity towards reagents. BioBond™ produces a very strong adhesion between the glass and the tissue section for subsequent incubations. BioBond™ coats the glass slide with a protective layer to minimise interaction of charged glass surface with reagents. This is also of particular importance for reproducibility of results because of the variations that occur between glass slides obtained from different sources and in different countries. It is particularly effective for use with severe incubating conditions such as those used in situ hybridisation.

**Simple Coating Procedure**
With BioBond™ there are no complicated procedures for coating slides. The following protocol will coat 100 or more slides. Wear disposable rubber gloves while handling BioBond™.

1. Load racks (metal or plastic) with slides. A rack of 25 at a time is most convenient.
2. Place the racks in a 2% solution of Decon detergent and leave soaking for 1 hour to clean.
3. Rinse thoroughly with tap water to remove all traces of Decon. If the local tap water is unclean or very hard then use distilled water.
4. Air dry the slides at room temperature or at 40°C. Cover to protect from dust.
5. Prepare a 2% solution of BioBond™ in acetone (2ml in 100ml). General purpose reagent will be sufficient.
6. Dip the rack of slides in BioBond™ solution for 4 minutes.
7. Rinse for 5 minutes in clean tap water or distilled water.
8. Air dry
9. Once the slides are completely dry store in a dust free environment until needed (usually in the original slide box).

The slides are then ready for mounting sections. They may be stored indefinitely, A 100ml solution of 2% BioBond™ will coat at least 100 slides by this method.

BioBond™ is suitable for all kinds of tissue specimens including paraffin wax or resin sections, cell smears, cytospins or cryostat sections. BioBond™ is supplied in 20ml unit volumes, sufficient to coat at least 1000 slides.

Return to:

- BioBond™ Tissue Adhesive Main Page
- SPI Supplies Chemicals for Microscopy
- SPI Supplies Catalog Table of Contents
- SPI Supplies Home Page

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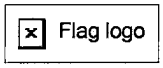
Worldwide Distributors, Representatives, and Agents 

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Hatfield, PA 19440
Tel #: 215-412-8400
Fax #: 215-412-8450
Fax #: 215-412-8451
E-Mail: sgkcck@aol.com

# ➤ BioBond Tissue Section Adhesive

### EMS Catalog #71304

### Introduction:

For immunohistochemistry the choice of specimen preparation is critical for the preservation of antigens in the sample. Of greatest importance in the preparation schedules are the specimen fixation and embedding. The protocol must satisfy the requirements for preservation of structural integrity and antigenicity.

### Why Coat Slides?

Having prepared the tissue specimen for immunolabelling it is then imperative to perform the incubations with a protocol designed to maximize the specific signal and minimize the background. Some incubation conditions may cause tissue sections to be removed from the glass slide. Typical tissue section adhesives such as poly-L-Lysine, Elmer's glue, chrome alum, etc. are not suitable for use with immunogold labeling because of the increased background caused by the attraction of gold particles to the adhesive on the slide.

In addition the surface of glass slides is uneven and is activated by the silicon tetrahedral structure. It therefore provides active sites for absorption of proteins or reactions with chemicals and reagents. It is therefore important to minimize this possibility by coating the surface with a material that is of low reactivity towards reagents.

BIOBOND produces a very strong adhesion between the glass and the tissue section for subsequent incubations. BIOBOND coats the glass slide with a protective layer to minimize interaction of charged glass surface with reagents. This is also of particular importance for reproducibility of results because of the variations that occur between glass slides obtained from different sources and in different countries. It is particularly effective for use with severe incubating conditions such as those used in situ hybridization.

### Simple Coating Procedure:

With BIOBOND there are no complicated procedures for the coating of the slides. The following protocol will coat 100 or more slides.

Wear disposable rubber gloves while handling BIOBOND.

- Load racks with slides. A rack of 25 at a time is most convenient .
- Place the racks in a 2% solution of Decon detergent and leave soaking for 1 hour to clean.
- Rinse thoroughly with tap water to remove all traces of Decon. If the local tap water is dirty or very hard use distilled water.
- Air dry the slides at room temperature or at 40°C. Cover to protect from dust.
- Prepare a 2% solution of Biobond in acetone(2ml in 100ml). General purpose reagent will be sufficient.
- Dip the rack of slides in BIOBOND solution for 4 minutes.
- Rinse for 5 minutes in clean tap water or distilled water.
- Air dry.
- Once the slides are fully dried store in a dust free environment until needed.

The slides are now ready for mounting sections. They may be stored indefinitely.

A 100ml solution of 2% BIOBOND will coat at least 100 slides.

BIOBOND is suitable for all kinds of tissue specimens including paraffin wax or resin sections, cell smears, cytospins, or cryostat sections.

### Online Ordering

BioBond Tissue Section Adhesive is available online from the EMS Catalog. For ordering or product information, click here.

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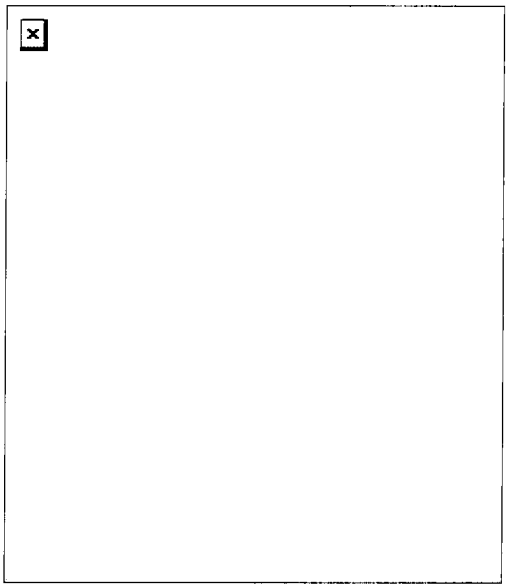
# EXHIBIT G

SPI Supplies

# Using LR White™ for Electron Microscopy

**Brought to you by the London Resin Company and SPI Supplies**

When using LR White™ embedding resin for dedicated electron microscopy, very few changes need to be made to the regime used for epoxy resin embedding. Every laboratory has its own individual embedding schedule but we have laid out here a "typical" schedule for LR White as guidance for its use.



**"Human Oral Epithelium, PTA stained"**

**Fixation:**
No change from normal fixation should be made, if EM only is required from the final blocks.

If however, good ultrastructure and a wide range of LM staining is required, then we have found that the use of freshly depolymerized paraformaldehyde (3 -4%) in a phosphate buffer pH 7.2 with 2½% w/v sucrose is the best compromise. Glutaraldehyde along and Karnovsky's glutaraldehyde- formaldehyde mixtures may lead to patching LM staining and some stains not working or giving "false positives" (e. g. PAS) whereas normal formalin fixation yields unacceptable EM ultrastructure.

For the dual LM/EM role, osmium tetroxide should be avoided due to its effect on many LM stains but 1% phosphotungstic acid (w/v) in the first absolute ethanol step of dehydration improves electron contrast without adversely affecting most LM stains. Osmium tetroxide may be used if the blocks are required for dedicated electron microscopy only.

**Dehydration:**
A graded ethanol series is the method of choice when embedding in LR White. Acetone acts as a radical scavenger in the resin system and therefore traces of acetone left in the tissue at curing can interfere with this polymerization. For this reason the use of graded acetone series and 2,2- dimethoxypropane (which generate acetone) are best avoided. If the use of 2 , 2-dimethoxypropane is considered vital we recommend either a protracted resin infiltration or washing the tissue with dry ethanol prior to infiltration in order to minimize the chance of acetone contamination of the final resin.

**Infiltration:**
The extreme low viscosity of LR White may be exploited by allowing the use of short infiltration times or large specimens **but not both**. A 1 mm cube of animal tissue will be adequately infiltrated in about 3 hours if 4-6 changes of LR White at 60° C are employed during this period. An overnight infiltration at room temperature, followed by two short changes of resin will often be more convenient, however. The long shelf life and low extraction rate of LR White allows specimens (perhaps reserve tissue) to be stored safely in resin for many weeks at 4° C if required. Larger blocks do require significantly longer infiltration times than small ones.

**Polymerization:**
Osmium tetroxide reacted tissues should not be "cold cured" with the accelerator. This process is strongly exothermic and the dark color of the tissue leads to a local heat accumulation which can cause local problems in and around the tissue. So we want to repeat this admonition: Do not use the accelerator with already osmium tetroxide stained tissue. Post staining with osmium tetroxide is quite acceptable, but only after polymerization has occurred.

If the tissue is not osmium tetroxide post-fixed then curing with LR White accelerator may be employed. As with curing blocks for light microscopy we recommend cooling the molds during polymerization, but there is no need to

exclude oxygen from the surface of the curing block.

Thermal curing should be used for osmicated specimens and may be used for all specimens. Here it is important to limit the contact of oxygen with the resin while polymerization occurs. The most convenient way of achieving this with capsule-type embedding is to use gelatin capsules, fill up to the brim and slide the other half of the capsule on.

If flat embedding is required for cutting orientation then the surface of the resin must be covered to prevent contact with oxygen. One convenient method is to utilize the JB-4-type molds and chucks, useful for light microscopy, and after polymerization the block may be sawed off the stub and the mold reused.

Polymerization time and temperature are fundamental to the physical characteristics of the final block, to a much higher degree than than with undercured epoxy systems.

We strongly recommend a temperature of 60° ± 2° for a period of 20-24 hours. Some ovens are not capable of controlling polymerization temperature so closely, and if faced with over brittle blocks, this is the first parameter to check.

LR White has extremely good powers of penetration and can penetrate and soften some low-density polyethylene capsules. This causes them to soften and collapse. Also polyethylene is not impermeable to oxygen and may allow enough contact with atmospheric oxygen to give the blocks an inhibited "tacky" surface. Both these problems may be overcome by the use of gelatin capsules (size 00 is similar to the popular polyethylene capsules size) and these are much cheaper and easier to seal during polymerization.

Resin may be used straight from the refrigerator and has a very low toxicity both in monomeric and polymerized state, unlike epoxies (see Proc. **Roy. Mic. Soc. 16**, Part 4, p. 265-271). The cold cure accelerator does have some toxic risk and contact with the skin and eyes should be avoided.

For cold curing the accelerator should be used at one drop per 10 ml of resin and this should cause the polymerization to occur between 10-20 minutes. If polymerization occurs faster than this, we recommend either more careful measuring of the one drop of accelerator or a higher volume of resin per drop of accelerator.

**Trimming and cutting:**
Trimming the block may be done with a jeweller's saw, razor blade or with a glass knife on the ultramicrotome as with epoxy resin blocks.

Cutting, too, may be performed in the same way as for an epoxy resin with glass or diamond knives. A typical cutting speed of about 1 mm per second is suitable.

**Section Staining:**
All the common section stains give good results on tissue embedded in LR White resin. Stains made up in ethanol or methanol should be avoided as these solvents soften the resin and may remove sections from grids. As an alternative to uranyl acetate, 1% phosphotungstic acid has proved a good general purpose stain, both as a block stain, as already mentioned, and as a section stain.

**In the electron microscope:**
An initial reduction in electron density may accompany the initial exposure of the resin to the beam. This is thought to represent a loss of water, imbibed from knife-boat or staining solutions. Thinning as such does not occur and specimens have been kept stationary under a 120 KV electron beam for 3 hours with no obvious signs of damage.

Skin Care and Safety Products: For hand protection, consideration should be given to the use of Skin Barrier Cream 222® and Skin Conditioner 212®.

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# ➤ LR White for Light Microscopy

### EMS Catalog #14380-14382

### Using LR White for Light Microscopy

Resin embedding for light microscopy provides greatly improved cellular definition compared to paraffin embedding, and for this reason is now widely used in diagnoses particularly of Renal disease, Lymphomas and bone marrow trephines, as well as research.

The acrylic resins currently used however are not suitable for E.M. and the epoxy resins used for E.M. are not easily stained for light microscopy. 'L.R. White' however can be used for both purposes and a lymph node for example (12 x 10 x 3 mm) can be processed, cut and stained for light microscopy, then the same block trimmed down, cut and stained for electron microscopy.

L.R. White can also be used for the histochemical demonstration of some of the more resistant enzymes and for the immunocytochemical demonstration of intracellular immunoglobulins.

For those laboratories already using an acrylic resin e.g. HEMA or Glycol Methacrylate no alteration need be made to the current processing schedule, but we have laid out here a 'typical' schedule for L.R. White as guidance for its use.

### Fixation

No change from normal fixation need be made if LM only is required from the final blocks (Neutral Buffered Formalin recommended). If however EM is required subsequent to LM then we have found the use of freshly depolymerized paraformaldehyde (3-4%) in a phosphate buffer pH 7.2 with 2.5% w/v sucrose is the best compromise. Glutaraldehyde-formaldehyde mixtures may lead to very pale staining with haematoxylin and patchy eosin, whereas normal formalin fixation gives unacceptable EM structure. For the dual LM/EM role osmium tetroxide should be avoided due to its effect on many LM stains but 1% phosphotungstic acid (w/v) in the first absolute ethanol step of dehydration improves electron contrast without adversely affecting most LM stains. If this does not provide adequate electron density then 'staining' of ultrathin sections can be carried out with osmium (a brief exposure to 1% aqueous osmium tetroxide or osmium tetroxide vapor on a copper grid) or lead citrate.

### Dehydration

A graded ethanol series is the method of choice when using L.R. White. Acetone acts as a radical scavenger in the resin system and traces of acetone left in the tissue at curing can interfere with polymerization.

### Infiltration

The extreme low viscosity of L.R. White allows the use of short infiltration times, but these will obviously depend on the size of the tissue. Infiltrated tissue will become translucent and sink to the bottom of the container.

A typical dehydration and infiltration schedule for a block (12 x 10 x 3 mm) on a mixer would be:

1. Two changes 70% alcohol 30 minutes each
2. Two changes Absolute alcohol 30 minutes each
3. Infiltrate with L.R. White at RT 2-3 changes 60 minutes each or leave overnight.

### Polymerization

Either heat or cold curing can be used for L.M., cold curing gives slightly better cutting and staining qualities. When cold curing it is important to cool the molds in a bath of cold water, during polymerization, to disperse the heat produced by the exothermic reaction, but it is not necessary to

exclude oxygen from the surface of the curing block.

Some polymerization problems have been experienced when embedding very flat pieces of tissue which stick to the base of the embedding mold. The way to avoid this is to smear the base of the mold with accelerator before adding mixing resin, and allow the tissue to sink to the base of the mold rather than applying pressure.

When thermal curing it is important to limit the contact of oxygen with the resin while polymerization occurs. The most convenient way of achieving this is to use gelatin capsules (EMS Catalog #70100) for small pieces of tissue. Fill up to the brim and slide the other half of the capsule on. For larger specimens the surface of the resin must be covered and one convenient method is to utilize the JB-4 type molds, one being used as a lid for another, or to polymerize in a nitrogen environment.

Polymerization time and temperature are fundamental to the physical character of the final block, to a much greater extent than with under cured epoxy systems.

We strongly recommend a temperature of 60°C ± 2°C for a period of 20-24 hours. Some ovens are not capable of controlling temperature so closely and it faced with over brittle blocks this is the parameter to check.

Resin may be used straight from the refrigerator and has a very low toxicity in both monomeric and polymerized states unlike epoxies (see Proc. Mic. Soc. (1981), 16, Pt.4, p. 265-271). The cold cure accelerator does have some toxic risk and contact with skin and eyes should be avoided.

For cold curing the accelerator should be used at one drop per 10 ml of resin and this should cause polymerization in 10-20 minutes. If polymerization occurs faster than this we recommend either more careful metering of the one drop of accelerator or a higher volume of resin per drop of accelerator.

## Cutting and Mounting

Although it is possible to cut L.R. White on a standard microtome with a steel knife the method of choice would be to use a heavy duty motorized microtome, and glass (Ralph type) knife.

L.R. White can cut as thin as 0.25 micron on some microtomes, but it is very difficult to obtain a satisfactory stain intensity with anything other than toluidine blue at this thickness, simply because there is so little tissue present in the sections.

For hematoxylin and eosin staining as well as most other routine stains we recommend sections of 2-3 micron. It is of course possible to cut thicker (up to 15 or 20 micron) if required.

Blocks can be cut dry, the sections picked up and floated out on 30-50% acetone on a hot plate @ 60-70°C.

To 20 ml acetone add 0.5 ml benzyl alcohol mix then make up to 50 ml with distilled water. A section adhesive such as egg albumin, can be added to this if required.

## Section Staining

Most routine stains give good results on tissue embedded in L.R. White resin using standard times and temperatures although it may occasionally be necessary to extend some staining times e.g. Methyl Green Pyronin. Stains made up in ethanol or methanol should be avoided as these solvents soften the resin and may remove sections from the slide. Dehydration of sections through graded alcohol after staining should also be avoided. Sections should be blotted, air dried and then mounted in a resinous mounting medium.

## Online Ordering

Using LR White for Light Microscopy is available online from the EMS Catalog. For ordering or product information, click here.

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# EXHIBIT H

# LR Gold™ Resin Systems

**Technical Data Sheet: Processing and Polymerization**

While tissue samples up to 5 x 5 x 5 mm have been successfully processed using the following schedule, it is recommended that tissue specimens no longer than 3 x 3 x 3 mm be used. Further, as a general rule the smaller the specimen the more efficient the impregnation. The thickness of the tissue is particularly important when polymerizing darkly colored tissue such as liver and spleen, because complete polymerization depends on the blue light from the light source being able to penetrate the full thickness of the tissue. Tissue used should be fresh and unfixed.

## Processing

Processing is performed in 10 ml vials with tight fitting lids on a rotary agitator at the temperatures listed in the table below. The fluids involved are stored in bulk at the sub-zero temperatures required. Also, it must be kept in mind, the mixed resin are sensitive to prolonged light exposure and are therefore stored in the dark and handled as infrequently as possible. We recommend the use of polyvinyl pyrollidone to protect unfixed tissue from osmotic changes during processing. We have used PVP with an approximate molecular weight of 44,000. This can be dissolved in methanol, water, or the London Resin Gold monomer. Concentrations of 50% w/v are possible in the methanol mixtures, however, at low temperatures, the higher viscosity is impractical. The following schedule shows the recommended PVP concentrations.

While this protocal yields very satisfactory results for LM work, it must be said, that the addition of PVP in different concentrations may further improve morphology, especially in the EM.

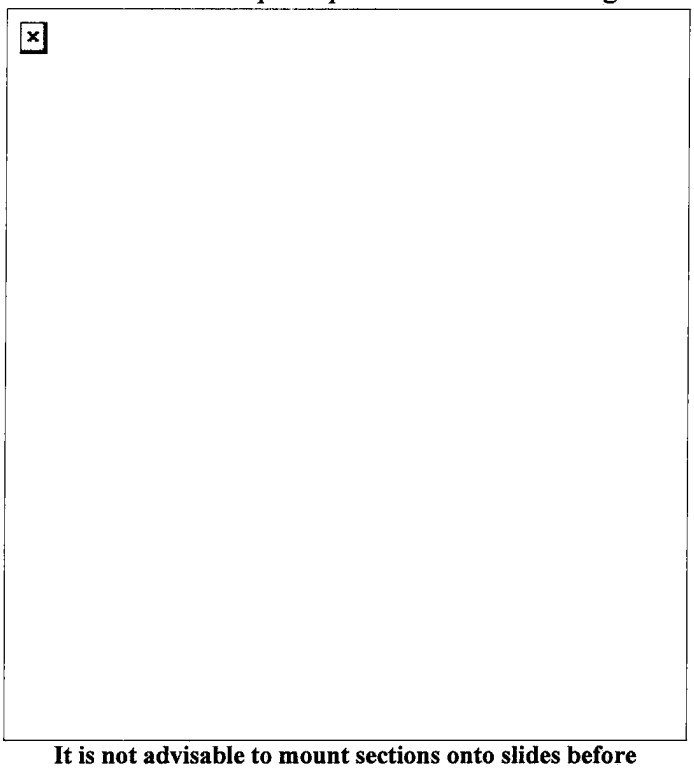
## Fresh Tissue

| | | |
|---|---|---|
| 50% methanol, 20% in PVP* | 0°C | 15 min. |
| 50% methanol, 10% in PVP | -25°C | 45 min. |
| 30% methanol, 10% in PVP | -25°C | 45 min. |

| | | |
|---|---|---|
| 50% LRGold monomer / 50% LRGold methanol, 10% in PVP | -25°C | 30 min. |
| 70% LRGold monomer / 30% LRGold methanol, 10% in PVP | -25°C | 60 min. |
| 100% LRGold monomer | -25°C | 60 min. |
| 100% LRGold monomer + initiator | -25°C | 60 min. |
| 100% LRGold monomer + initiator | -25°C | Overnight |
| 100% LRGold monomer + initiator | -25°C | 20-25 hours polymerization |
| * 20g PVP in 100ml methanol | | |

## Polymerization

The addition of a light sensitive initiator is needed in order to polymerize the resin and we recommend Benzil, an alpha-diketone, at a concentration of 0.1% w/v. The principle is shown in the diagram below.



**It is not advisable to mount sections onto slides before reacting, since this involves heat and would be deleterious to the unfixed proteins. The section can of course be mounted in the usual way after enzyme histochemistry or immunocytochemistry has been carried out.**

The gelatin capsules are 00 size and the plastic support is a modified heamagglutination tray. The bulb involved here is a *Thorn projector lamp* (AI/209 FDX, 12V 100W). We have found that 7 to 9 V will induce solidification within 24 hours. As with many acrylic resins oxygen will inhibit polymerization, therefore, the capsules should be filled completely and lids fitted. Paper labels can be inserted into the capsules. Nine capsules (3 x 3) may be polymerized at anyone time. If the upper surface is still soft after 24 hours, it can be trimmed off or hardened in daylight for a few hours prior to peeling off the gelatin. Once polymerized, the blocks need not be stored cold, but storage in the cold may prolong the activity of some enzymes.

Enzyme histochemistry performed to date using this resin has involved conventional reagents, times of reaction and

temperatures (see Thompson and Germain, Histochemical Journal Vol. 15, No. 12, December 1983).

For room temperature polymerization, using a peroxide/amine cure, add to pure LRGold either 1% of dry benzoyl peroxide or, more safely, 1.5% of benzoyl peroxide paste (60% in dibutyl phthalate). Infiltrate with pure LRGold solution, adding the peroxide mix just prior to polymerization. To reduce the heat of curing, cool the mold in ice water. To accelerate the cure, add 1 drop of LRWhite accelerator to 20ml LRGold resin/benzoyl peroxide mixture. To U.V. light cure, add benzoin methyl ether. The precise concentration of the ether will depend on the power and emission spectrum of your UV lamp. However, a useful starting concentration would be 0.5%.

The cross link density of the final resin is important. If stains are not penetrating sufficiently quickly, reduce the benzil concentration rather than the light intensity or exposure time.

The LRGold blocks, following polymerization, can be stored, handled and cut at room temperature. Cutting should preferably be done using a motorized microtome and a glass knife. Sections may be cut dry, picked up and placed free-floating into incubating medium or buffer wash for enzyme histochemistry or immunocytochemistry.

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# ➤ LR Gold Processing and Polymerisation

### EMS Catalog #14370

### Tissue

Tissue samples up to 5 x 5 x 5 mm have been successfully processed using the following schedule. However, it is recommended that tissue specimens of maximum 2 x 3 x 3 mm are used and as a general rule the smaller the specimen the more efficient the impregnation. The thickness of the tissue is particularly important when polymerising darkly coloured tissue such as liver and spleen, because complete polymerisation depends on the blue light from the light source being able to penetrate the full thickness of the tissue. Tissue used is fresh and unfixed.

### Processing

Processing is performed in 10 ml vials with tight fitting lids on a rotary agitator. The fluids involved are maintained in bulk at the sub-zero temperatures required. Also, the final resins, it must be remembered, are sensitive to prolonged light exposure and are therefore stored in the dark and handled as infrequently as possible. We recommend the use of polyvinyl pyrollidone to protect unfixed tissue from osmotic changes during processing. We have used PVP with an approximate molecular weight of 44,000. This can be dissolved in methanol, water and the London Resin Gold monomer. Concentrations of 50% w/v are possible in the methanol mixtures, however, at low temperatures the resulting viscosity is impractical. The following schedule shows the PVP concentrations recommended, the resulting LM work being very satisfactory. It must be said however, that the addition of PVP in different concentrations may further improve morphology especially in the EM.

#### Fresh Tissue

| | | |
|---|---|---|
| 50% methanol + 20% PVP | 0°C | 15 min |
| 70% methanol + 20% PVP | -25°C | 45 min |
| 90% methanol + 20% PVP | -25°C | 45 min |
| 50% LR Gold monomer/50% methanol + 10% PVP | -25°C | 30 min |
| 70% LR Gold monomer/30% methanol + 10% PVP | -25°C | 60 min |
| 100% LR Gold monomer | -25°C | 60 min |
| 100% LR Gold monomer + initiator | -25°C | 60 min |
| 100% LR Gold monomer + initiator | -25°C | overnight |
| 100% LR Gold monomer + initiator | -25°C | 20 - 25 hour polymerisation |

### Polymerisation

The addition of a light sensitive initiator is needed in order to polymerise the resin and we recommend BENZIL, an alphadiketone, at a concentration of 0.1% w/v. The principle is shown in the diagram. The gelatin capsules are 00 size and the plastic support is a modified heamagglutination tray. The bulb involved here is a Thorn projector lamp (A1 / 209 FDX, 12V, 100W). We have found that 7 to 9V will cause solidification within 24 hours. Like many acrylic resins oxygen will inhibit polymerisation, therefore the capsules are filled completely and lids fitted. Paper labels can be inserted into the capsules. 9 capsules (3 x 3) may be polymerised at any one time. If the upper surface is still soft after 24 hours this can be trimmed off or hardened in daylight for a few hours prior to peeling off the gelatin. The blocks once polymerised need not be stored cold, however it may prolong the activity of some enzymes to do so.

The enzyme histochemistry performed to date using the resin has involved conventional reagents, times of reaction and temperatures (see Thompson and Germain, Histochemical Journal Vol. 15, No. 12, December 1983.)

For room temperature polymerisation, using a peroxide/amine cure, add to pure LRGold either 1%

For room temperature polymerisation, using a peroxide/amine cure, add to pure LRGold either 1% of dry benzoyl peroxide or, more safely, 1.5% of benzoyl peroxide paste (60% in dibutyl phthalate).

Infiltrate with pure LRGold solution adding only the peroxide mix prior to polymerisation. To reduce curing exotherm, cool mould in ice water. To accelerate cure, add 1 drop of LRWhite accelerator to 20ml LR Gold resin/benzoyl peroxide mixture. To UV light cure LRGold, add benzoin methyl ether. The precise concentration will depend on the power and emission spectrum of your UV lamp. However, a useful starting concentration would be 0.5%.

The cross link density of the final resin is important. If stains are not penetrating sufficiently quickly, reduce the benzil concentration rather than the light intensity or exposure time.

## Sectioning and Mounting

The LR Gold blocks, following polymerisation, can be stored, handled and cut at room temperature. Cutting should preferably be done using a motorised microtome and glass knife. Sections may be cut dry, picked up and placed free-floating into incubating medium or buffer for enzyme histochemistry or immunocytochemistry.

It is not advisable to mount sections onto slides before reacting, since this involves heat and would be deleterious to the unfixed proteins. The section can of course be mounted in the usual way after enzyme histochemistry or immunocytochemistry has been carried out.

## Online Ordering

LR Gold Embedding Kit is available online from the EMS Catalog. For ordering or product information, click here.

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# EXHIBIT I

# SPI Diffraction Standards, TEM

**Principal lattice spacings for thallous chloride and aluminum**

## General Instructions

Diffraction pattern ring diameters can be simply related to the lattice plane spacings in the crystal used as the specimen.

For a lattice spacing d, in a microscope of effective camera length L and operating at an accelerating voltage corresponding to a wavelength *, the diffraction ring diameter is r where *L = dr

It is not easy to measure the actual value of L or know the precise value of accelerating voltage. However, without knowing the exact values of * and L, one can determine an unknown lattice spacing by measurement of r, if the instrument has first been calibrated with a known substance.

This can best be done by using a polycrystalline material of known lattice spacings, so that continuous diffraction rings are available for measurement. The ring diameters must all be measured in the same plane relative to the microscope (to avoid any errors due to ellipticity of the pattern).

Furthermore, the calibrating ring diameter chosen should be similar to that required to be calibrated since there may be distortion of the relative ring diameters due to residual barrel or pincushion distortion in the projector lens system.

**SPI #02856-AB Evaporated Thallous Chloride**

Thallous chloride has a simple cubic structure with a = 0.3842 nm.

**If N = h2+ k2+ l2, the principal lattice spacings are as follows:**

| N | Lattice Spacings (nm) |
|---|---|
| 1 | 0.384 |
| 2 | 0.272 |
| 3 | 0.249 |
| 4 | 0.192 |
| 5 | 0.172 |
| 6 | 0.157 |
| 8 | 0.136 |
| 9 | 0.128 |
| 10 | 0.121 |

The thallous chloride is evaporated on to a carbon substrate to form a polycrystalline layer.

**SPI #02857-AB Evaporated Aluminium**

Aluminium has a face centred cubic structure, with a = 0.4041 nm.

**If N = h2+ k2+ l2, the principal lattice spacings are as follows:**

| N | Lattice Spacings (nm) |
|---|---|
| 3 | 0.234 |
| 4 | 0.202 |
| 8 | 0.143 |
| 11 | 0.122 |

| | |
|---|---|
| 12 | 0.117 |
| 16 | 0.101 |

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# ➤ Molybdenum Oxide Crystals

### EMS Catalog #80046

### Diffraction Standard

### General Instructions

Diffraction pattern ring diameter can be simply related to the lattice plane spacing in the crystal used as the specimen.

For a lattice spacing d, in a microscope of effective camera length L and operating at an accelerating voltage corresponding to a wavelength l , the diffraction ring diameter is r where l L = dr

It is not easy to measure the actual value of L or know the precise value of accelerating voltage. However, without knowing the exact values of l and L, one can determine an unknown lattice spacing by measurement of r, if the instrument has first been calibrated with a known substance.

This can best be done by using a polycrystalline material of known lattice spacing, so that continuous diffraction rings are available for measurement. The ring diameters must all be measured in the same plane relative to the microscope (to avoid any errors due to ellipticity of the pattern).

Furthermore, the calibrating ring diameter chosen should be similar to that required to be calibrated since there may be distortion of the relative ring diameters due to residual barrel or pincushion distortion in the projector lens system.

This test specimen is most useful to determine the rotation between a diffraction pattern and the selected area image.

Select a thin crystal not overlaid by others, so that a clear Lauer diffraction pattern is obtained.

Starting from the diffraction pattern, change the strength of the diffraction lens until each diffraction spot shows a small image of the crystal. It will be possible to determine the sense of the rotation of the image as the magnification is increased. Check whether there is an image inversion between the diffraction position and the selected area magnification.

The actual magnitude of the rotation angle between the crystal and its pattern can be determined by recording both image and diffraction pattern on a single plate. The correct rotation angle between pattern and image can then be determined by taking into account the sense of rotation and any image inversion.

### Online Ordering

Molybdenum Oxide Crystals are available online from the EMS Catalog. For ordering or product information, click here.

# EXHIBIT J

# Tempfix™ Mounting Adhesive for SEM Samples

**Instructions for Use**

Warm up the aluminum mount on the hot plate to approximately 120° C. Apply a small amount of Tempfix and smooth it over the top of the SEM mount with a spatula. We would recommend a PTFE coated spatula because of its well known non-stick characteristics.

Remove any excess Tempfix. Once the resin is "drawn down" on the surface of the SEM mount, so long as the mount is protected from falling dust, the mounts can be stored in this state indefinitely.

For the SEM examination of dry or relatively dry powder specimens, sprinkle the powder onto one of the Tempfix-coated aluminum SEM mounts. Warm up the mount and Tempfix film for about ten to fifteen seconds to roughly 40° C, using the hot plate. Then remove the mount and cool it on a metal block.

For samples especially prone to heat damage, even at 40° C, a variation of the technique is to perform the mounting process, instead of directly on an aluminum SEM mount, using an aluminum sheet of thickness 0.5 to 1.0 mm. Use of such a sheet permits a more rapid cooling of the heated sample, minimizing any possibility for heat induced damage to the specimen. Once this is done, a small piece can be cut out and glued down to a conventional SEM mount, using nothing more exotic that our 5-minute epoxy.

Another possibility is the use of "heavy" gage aluminum foil but some might find it less convenient to use in this way.

Any specimen prepared by any of these methods can be placed in a sputter coater and metallized using gold, palladium, silver, platinum or an alloy of gold and palladium (60% gold/40% palladium). Of course, we would recommend using gold in most instances because, having the lowest work function of the different alternatives, it will sputter faster than any other metal or alloy. This minimizes the amount of time a possible delicate sample is exposed to the radiant heat from the plasma of the sputter coater. Or if you are seeking maximum resolution using a FESEM, it can be coated with amorphous osmium metal in one of the OPC family of osmium plasma coaters.

Or alternatively, if the sample is destined for EDS analysis, it can be placed in a carbon coater and coated for x-ray analysis (EDS).

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## Technical Data Sheets



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# ➤ Tempfix Mounting Adhesive

### EMS #12668

Warm up the aluminum mount on the hot plate to approximately 120°C. Apply a small amount of Tempfix and smooth it over the top of the SEM mount with a spatula. We would recommend a PTFE coated spatula because of its well known non-stick characteristics.

Remove any excess Tempfix. Once the resin is "drawn down" on the surface of the SEM mount, so long as the mount is protected from falling dust, the mounts can be stored in this state indefinitely.

For the SEM examination of dry or relatively dry powder specimens, sprinkle the powder onto one of the Tempfix-coated aluminum SEM mounts. Warm up the mount and Tempfix film for about ten to fifteen seconds to roughly 40°C, using the hot plate. Then remove the mount and cool it on a metal block.

For samples especially prone to heat damage, even at 40°C, a variation of the technique is to perform the mounting process, instead of directly on an aluminum SEM mount, using an aluminum sheet of thickness 0.5 to 1.0 mm. Use of such a sheet permits a more rapid cooling of the heated sample, minimizing any possibility for heat induced damage to the specimen. Once this is done, a small piece can be cut out and glued down to a conventional SEM mount, using nothing more exotic than Mascot Insttant Adhesive (catalog 72587-01 .

Another possibility is the use of "heavy" gage aluminum foil but some might find it less convenient to use in this way.

Any specimen prepared by any of these methods can be placed in a sputter coater and metallized using gold, palladium, silver, platinum or an alloy of gold and palladium (60% gold/40% palladium). Of course, we would recommend using gold in most instances because, having the lowest work function of the different alternatives, it will sputter faster than any other metal or alloy. This minimizes the amount of time a possible delicate sample is exposed to the radiant heat from the plasma of the sputter coater.

Or alternatively, if the sample is destined for EDS analysis, it can be placed in a carbon coater and coated for x-ray analysis (EDS).

### Online Ordering

Tempfix Mounting Adhesive is available online from the EMS Catalog. For ordering or product information, click here.

# EXHIBIT K

# SIRA Calibration Specimen Set

**Complete Use Instructions**

## General Information about the SIRA Specimen

These high quality calibration specimens provide a means of testing scanning electron microscopes, scanning transmission electron microscopes (in the secondary electron mode), and electron probe microanalysis systems particularly for magnification accuracy above 20X, for tilt specifications on the stage, for image distortion and for the measurement of the depth of field. However they are also usable for tests of stage stability, centering of stub rotation, checks for symptoms of electrical and mechanical interference, etc.

Two metal (resin-backed) grating replicas, one of a 19.7 and the other of a 2160 lines/mm original are mounted on sample mounts and gold coated. Each replica contains lines in two orthogonal directions with line frequencies guaranteed to be within 1% of the stated figure on delivery. The specimens are flat with a usable area of over 60 square mm, and were originally developed by SIRA EM Techniques Group.

For more detailed information, the reader is referred to an early research note: "A New Magnification Test Specimen for SEMs" by I. M. Watt and N. A. Wright, Metron (UK) 3, No 6, 153-156 (1971).

## Using the Specimen

### 1. Magnification Calibration

The 19.7 lines/mm sample (Figure 1) is suitable from about 20X to 1600X magnification on the instrument "CRT"; the finer specimen ruled to 2160 lines/mm (Figure 2) can be used from 1000X and up. It is essential that the user first ensures that the image distortion levels are within acceptable tolerances at the magnification levels of interest before embarking on the magnification calibration. In addition, on some instruments it is necessary to work the magnification calibration routine in well defined and reproducible operational conditions (e.g. working distances, KV). Further it is better to work with zero tilt in the direction used for calibration. For example, Figures 1 and 2 were recorded with X-tilt on the stage but zero Y tilt. The calibration was performed using only the vertical lines which are not disturbed by the X-tilt. Magnification calibration routines should include checks on recorded images since the recording video display unit (CRT) may give a different magnification than the visual CRT.

The 19.7 lines/mm sample, use from about 20-1600X The 2160 lines/mm sample, use from about 1000X and upwards

### 2. Calibration of the stage tilt controls

At normal incidence of the electron probe on the specimen (zero X- and Y- tilt), the unit cells in the pattern are square. They become rectangular as the specimen plane is inclined to the electron-optical axis.

Figure 3 shows this effect for the case of the X-tilt, that is, tilt around the Y-axis. The ratio of short to long dimensions of the rectangle is the cosine of the actual tilt angle provided that the tilt axis is parallel to one set of the ruling. The coarse (19.71 lines/mm) sample is recommended for tilt-angle calibration.

Demonstration of the effect

of X-tilt around the Y- axis

**3. Observation of image distortion in the microscope**

The microscopist might want to measure and/or correct the degree of image distortion at various working distances, magnifications, and operational KV levels, normal incidence Figure 4 is recommended. It is also important to remember that distortions arise all along the imaging chain to the recording CRT. Hence it should not be forgotten that recorded images are important at this point and should never be over looked.

Measure and correct the degee of distortion
at different working distances, magnifications,

and KV settings.

**4. Measurement of depth of field**

Use a moderate (30 - 40°) X-tilt to demonstrate or check the depth of field by assessing the Z-depth for good focus. The depth of field is higher when the final aperture is smaller, when the working distance is longer, and when the SEM is operated in the lower magnification ranges.

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# ➤ General Information about the SIRA Specimen

### EMS Catalog #79505-02

These high quality calibration specimens provide a means of testing scanning electron microscopes, scanning transmission electron microscopes (in the secondary electron mode), and electron probe microanalysis systems particularly for magnification accuracy above 20X, for tilt specifications on the stage, for image distortion and for the measurement of the depth of field. However they are also usable for tests of stage stability, centering of stub rotation, checks for symptoms of electrical and mechanical interference, etc.

Two metal (resin-backed) grating replicas, one of a 19.7 and the other of a 2160 lines/mm original are mounted on sample mounts and gold coated. Each replica contains lines in two orthogonal directions with line frequencies guaranteed to be within 1% of the stated figure on delivery. The specimens are flat with a usable area of over 60 square mm, and were originally developed by SIRA EM Techniques Group.

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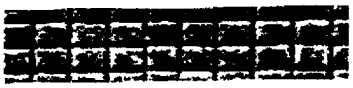
### Using the Specimen

#### 1. Magnification Calibration

The 19.7 lines/mm sample (Figure 1) is suitable from about 20X to 1600X magnification on the instrument " CRT "; the finer specimen ruled to 2160 lines/mm (Figure 2) can be used from 1000X and up. It is essential that the user first ensures that the image distortion levels are within acceptable tolerances at the magnification levels of interest before embarking on the magnification calibration. In addition, on some instruments it is necessary to work the magnification calibration routine in well defined and reproducible operational conditions (e.g. working distances, KV). Further it is better to work with zero tilt in the direction used for calibration. For example, Figures 1 and 2 were recorded with X-tilt on the stage but zero Y tilt. The calibration was performed using only the vertical lines which are not disturbed by the X-tilt. Magnification calibration routines should include checks on recorded images since the recording video display unit ( CRT ) may give a different magnification than the visual CRT .

The 19.7 lines/mm sample, use from about 20-1600X

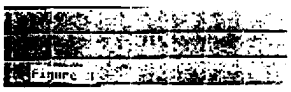


The 2160 lines/mm sample, use from about 1000X and upwards

#### 2. Calibration of the Stage Tilt Controls

At normal incidence of the electron probe on the specimen (zero X- and Y- tilt), the unit cells in the pattern are square. They become rectangular as the specimen plane is inclined to the electron-optical axis.

Figure 3 shows this effect for the case of the X-tilt, that is, tilt around the Y-axis. The ratio of short to long dimensions of the rectangle is the cosine of the actual tilt angle provided that the tilt axis is parallel to one set of the ruling. The coarse (19.71 lines/mm) sample is recommended for tilt-angle calibration.



Demonstration of the effect

of X-tilt around the Y- axis

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### 3. Observation of image distortion in the microscope

The microscopist might want to measure and/or correct the degree of image distortion at various working distances, magnifications, and operational KV levels, normal incidence Figure 4 is recommended. It is also important to remember that distortions arise all along the imaging chain to the recording CRT . Hence it should not be forgotten that recorded images are important at this point and should never be over looked.


Measure and correct the degee of distortion at different working distances, magnifications,

and KV settings.

### 4. Measurement of depth of field

Use a moderate (30 - 40 o ) X-tilt to demonstrate or check the depth of field by assessing the Z-depth for good focus. The depth of field is higher when the final aperture is smaller, when the working distance is longer, and when the SEM is operated in the lower magnification ranges.

## Online Ordering

SIRA Specimens are available online from the EMS Catalog. For ordering or product information, click here.

 

# EXHIBIT L

SPI Supplies is the source!

# Biomount™ Tissue Section Mounting Medium

**Reduces the rate of fading of immunogold/silver reagents in sections on glass slides**

**Introduction:**
Biomount™ reduces fading of immunogold/silver signals in sections on glass slides. It is suitable for both resin and wax embedded tissue sections.

Some mounting media oxidize rapidly on exposure to air, forming carboxyl groups. This may be especially so when sections have been cleared in a solution containing aldehyde groups. It is sometimes observed that the immunogold / silver stain fades after a few weeks, or even in a shorter time, from sections that have been mounted with these media under cover slips. The silver is still present, but has formed translucent silver carboxylate salts. Visibility can be retrieved by removing the cover slip and washing in xylene (xylol) and then immersing the slide in photographic developer, but this is a tedious procedure. Biomount prevents this fading because of its low oxidizing properties.

Biomount is miscible with xylene and may be applied to sections on slides in the normal manner following dehydration and immersion in xylene. Labelling will retain its intensity and contrast following the use of Biomount™. Sections that have been counterstained with normal stains or with the UNICRYL™ Staining Kit will also retain their color intensity.

**Product packaging:**
100 ml standard bottles and 30 ml bottle size

**Shipping information:**
UN #1993 Class 3 Flammable Liquid Packing Group: III

| | | Size | SPI # | Each | | In Stock |
|---|---|---|---|---|---|---|
| | | 30 ml | **04766-DA** | $ 43.22 | Add to cart | No |
| | | 100 ml | **04766-MB** | 55.28 | Add to cart | No |

Storage conditions: Room temperature
Hazardous from the standpoint of shipping

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# ➤ BIOMOUNT™

### EMS Catalog #17894

### Tissue Section Mounting Medium:

BIOMOUNT™ reduces fading of immunogold/silver signals in sections on glass slides. It is suitable for both resin and wax embedded tissue sections.

Some mounting media oxidise rapidly on exposure to air, forming carboxyl groups. This may be especially so when sections have been cleared in a solution containing aldehyde groups. It is sometimes observed that the immunogold / silver stain fades after a few weeks, or even in a shorter time, from sections that have been mounted with these media under cover slips. The silver is still present, but has formed translucent silver carboxylate salts. Visibility can be retrieved by removing the cover slip and washing in xylene (xylol) and then immersing the slide in photographic developer, but this is a tedious procedure. BIOMOUNT™ prevents this fading because of its low oxidising properties.

BIOMOUNT™ is miscible with xylene and may be applied to sections on slides in the normal manner following dehydration and immersion in xylene. Labelling will retain its intensity and contrast following the use of BIOMOUNT™. Sections that have been counterstained with normal stains or with the UNICRYL STAINING KIT will also retain their colour intensity.

### Online Ordering

BIOMOUNT™ is available online from the EMS Catalog. For ordering or product information, click here.

 